

One-pot lyophilized SHERLOCK assay

HD Helena de Puig JC James J. Collins HD Helena de Puig DN Devora Najjar

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An abbreviated version of this protocol was published in Science Advances in Aug 2021

Minimally instrumented SHERLOCK (miSHERLOCK) for CRISPR-based point-of-care diagnosis of SARS-CoV-2 and emerging variants

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Detailed protocol

Prepare lyophilized one pot SHERLOCK pellets:

Reagent manufacturers and further instructions are listed in the methods section in the Science Advances publication

1 - Prepare one-pot SHERLOCK solution using the following components into a 0.2mL PCR tube and mix well:

- 1 - TwistAmp Basic pellet (Twist Dx)
- 2 - 0.24uL of forward and reverse RPA primers each from a 100uM stock concentration
- 3 - 30uL of rehydration buffer (33mM HEPES pH 6.8, 100mM NaCl, 8.3% PEG)
- 4 - 2.5uL of Protoscript reverse transcriptase from a 100uM stock concentration
- 5 - 0.25uL of Ambion RNase H from a 10U/uL stock concentration
- 6 - 0.5uL of ssDNA probe
- 7 - 0.2uL of EnGen Lba Cas12a from a 50uM stock concentration
- 8 - 0.2uL of gRNA solution from a 100uM stock concentration
- 9 - 5uL of 10x NEB 2.1 buffer
- 2 - Punch a hole into the 0.2mL PCR tube cap using an 18 Gauge needle
- 3 - Snap freeze the above PCR tube in liquid nitrogen
- 4 - Quickly re-open snap-frozen PCR tube and add 2.5uL of Magnesium acetate from a 280mM stock concentration
- 5 - Snap freeze the PCR tube again
- 6 - Lyophilize the snap-frozen PCR tube for 6 hrs

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. de Puig, H. , Collins, J. , de Puig, H. and Najjar, D. (2021). One-pot lyophilized SHERLOCK assay. Bio-protocol Preprint. bio-protocol.org/prep1344.
2. Puig, H. D., Lee, R. A., Najjar, D., Tan, X., Soekensen, L. R., Angenent-Mari, N. M., Donghia, N. M., Weckman, N. E., Ory, A., Ng, C. F., Nguyen, P. Q., Mao, A. S., Ferrante, T. C., Lansberry, G., Sallum, H., Niemi, J. and Collins, J. J.(2021). Minimally instrumented SHERLOCK (miSHERLOCK) for CRISPR-based point-of-care diagnosis of SARS-CoV-2 and emerging variants. Science Advances 7(32). DOI: [10.1126/sciadv.abh2944](https://doi.org/10.1126/sciadv.abh2944)

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